A STUDY OF rDNA MAGNIFICATION PHENOMENON IN A REPAIR-RECOMBINATION DEFICIENT MUTANT OF *DROSOPHILA MELANOGASTER*

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Manuscript received December 21, 1981
Revised copy accepted April 26, 1982

ABSTRACT

The rDNA magnification process consists of a rapid and inheritable rDNA increase occurring in bobbed males: in a few generations the *bb* loci acquire the wild-type rDNA value and reach a bb^+ phenotype.——We have analyzed the rDNA magnification process in the repair-recombination-deficient mutant *mei9*^{a}, both at the phenotypical and rDNA content levels. In *mei9*^{a} *bb* double mutants the recovery of bb^+ phenotype is strongly disturbed and the rDNA redundancy value fails to reach the wild-type level. The strong effect of this meiotic mutation on rDNA magnification suggests a close relationship between this phenomenon and the repair-recombination processes.

THE bobbed (*bb*) locus of *D. melanogaster* maps proximal to the centromeres on the X chromosome and on the Y short arm. Wild-type (bb^+) loci consist of a cluster of 150–250 tandemly repeated genes for ribosomal RNA (for a review see RITOSSA 1976). The rRNA genes have an additive effect, that is the phenotype depends on the total number of the genes carried by both the X and Y chromosomes. The bb phenotype reduction of thickness and length of bristles, etched abdominal cuticle, delayed hatching time (LINDSLEY and GRELL 1968) arises when the diploid (i.e., X plus X, or X plus Y) number of rDNA repeats are below a certain value, usually about half of the stock rDNA content. Bobbed loci show a remarkable random instability of redundancy, and several mechanisms capable of changing rRNA gene multiplicity have been demonstrated. In addition to meiotic unequal crossing-over within the bobbed loci (SCHALET 1969), somatic gene compensation (TARTOF 1971; SPEAR and GALL 1973) and rDNA magnification (RITOSSA 1968; BONCINELLI *et al.* 1972; MALVA *et al.* 1972) have been described. Magnification occurs in males of bobbed phenotype with several possible genotypes: Xbb/Ybb^-; $Xbb/0$; $X_{NO}-/Ybb$; $\overline{XY}bb/0$; Xbb/Ybb. When an X chromosome carrying a partial or a total deletion of the *bb* locus is kept in combination with a Y chromosome also deleted in rDNA, a sudden and heritable increase of the rRNA gene number is observed. Males of this first generation, called premagnified (*pre*), still exhibit a strong bobbed phenotype with respect to their level of rDNA redundancy. This observation and other data (RITOSSA *et al.* 1971; GRAZIANI and GARGANO 1976) suggest that the new rDNA copies synthesized at this stage are not fully functional. If premagnified males are backcrossed with suitable females in order to maintain the same

genotype for two or more generations (called m_1, m_2 ...m_x), the multiplicity of rRNA cistrons reaches the wild-type level and the bb$^+$ phenotype reaches full penetrance. If, instead, a newly magnified *bb* locus (*pre*, m_1, m_2) is kept with a *bb*$^+$ chromosome, its rDNA redundancy decreases until it reaches a bobbed condition and this phenomenon is called "reversion" (*rev* locus) (RITOSSA 1968; BONCINELLI *et al.* 1972; LOCKER 1976). To account for such an observation, a model was proposed for magnification, involving synthesis in meiotic tissues of rDNA genes not fully integrated in the chromosomes (RITOSSA 1972). The fact that the capability to revert decreases in successive generations supports the idea that in a step process a certain percentage of the newly synthesized copies become integrated into the chromosome. The presence of circular rDNA copies has been demonstrated in excised testes from premagnified males (GRAZIANI, CAIZZI and GARGANO 1977), and in the same tissues an excess of rDNA content with respect to soma has been reported (LOCKER and MARRAKECHI 1977).

Genetic combinations undergoing rDNA magnification show a 20-fold increase in the crossing-over frequency between X and Y chromosomes at the *bb* locus relative to males not undergoing magnification, where crossing over is practically absent. Simultaneous integration of extrachromosomal circular rDNA copies in both the X and Y chromosomes has been evoked to account for such recombination events (RITOSSA 1973). To test the molecular basis of such a model and to clarify the mechanisms involved in the production and integration of the rDNA extracopies we have analyzed the magnification process in the meiotic *mei9*^{a} mutant, which has been described to be defective in the exchange process of meiosis (BAKER and CARPENTER 1972; for review see BAKER and HALL 1976), and in repair replication of DNA damages induced by UV and X ray (BOYD, GOLINO and SETLOW 1976; BAKER *et al.* 1976; NGUYEN and BOYD 1977).

Data reported in this paper point out the existence of an interrelationship between the repair-recombination processes and rDNA magnification.

MATERIAL AND METHODS

Drosophila melanogaster *stocks:* All stocks were grown at 21° ± 0.5°. Wild-type Canton S flies were originally from the University of Pavia. The stock *C(1)RM, l(1)sc^{J1}v bb/B^s*Y *bb y$^+$ l(1)sc^{J1+}, In(l)sc^{4}y sc^4/B^s*Y *bb y$^+$l(1)sc^{J1+}* was from the University of Bari.

y$^+$, Y^{s}InEN y x Y^l*y$^+$, C(1)RM, y^{2}su w^{a}w^{a}bb* and *w^{a}bb^l/w^{a}bb^l/B^s*Y, *w^{a}bb^l/B^s*Y were originally derived from the Oak Ridge collection. The *Ybb$^-$* chromosome was from the stock *C(1)RM, y vf/ Ybb$^-$* and *w sn/Ybb$^-$* from the Philadelphia collection. *y mei9^a/y$^+$*Y, *C(1)DX, y f/y$^+$*Y, *spa^{pol}/spa^{pol}* and *y pn cv v f y$^+$/B^s*Y, *C(1)DX/B^s*Y were kind gifts of B. BAKER.

Selection of the B^{s}Y bb y$^+$l(1)sc^{J1+} *chromosome:* A *B^s*Y *bb y$^+$l(1)sc^{J1+}* chromosome carrying a wider rDNA deletion was selected out of the *C(1)RM, l(1)sc^{J1}v bb/B^s Y bb y$^+$l(1)sc^{J1+}, In(1)sc^{4}y sc^4/ B^s*Y *bb y$^+$l(1)sc^{J1+}* stock. Single males from the stock were mated to heterozygous *w^{a}bb^l* females (first cross) to select for more severe bb phenotype of the *w^{a}bb^l/B^s*Y *bb y$^+$l(1)sc^{J1+}* male progeny. After 3 days the male parents of the first cross are singly mated with *C(1)RM, y v f/Y bb$^-$* females (second cross) and 2–3 days later the same males are crossed again with wild-type Canton S females (third cross). Virgins were taken from the progeny of the second cross and mated with the male progeny of the third cross, to put the Y *bb* selected chromosome in a stable stock condition, avoiding any rDNA magnification step. The *C(1)RM, y v f/B^s Y bb y$^+$ l(1)sc^{J1+}* females of this stock were used in the magnification scheme.

rDNA magnification scheme: Experimental schedule of crosses performed to study the magnification process in *mei9^a* mutant.

According to HENDERSON and RITOSSA (1970) F_1 males (pre-males) are crossed with females identical to their mothers to maintain the same genotype through succeeding generations (m_1 to m_7)

P ♂♂ *y mei9*^{a} *bb*/*y*$^+$*Y* × ♀♀ *C(1)RM, y v f*/*B*^{s}*Y bb y*$^+$*l(1)sc*$^{J1+}$
F_1 ♂♂ *y mei9*^{a} *bb*/*B*^{s}*Y bb y*$^+$*l(1)sc*$^{J1+}$ × ♀♀ *C(1)RM, y v f*/*B*^{s} *Y bb y*$^+$ *l(1)sc*$^{J1+}$ pre generation
F_2 m_1 generation; established stock
F_8 m_7 generation

Phenotype classification: In all crosses examined the male offspring were classified, as previously reported (BONCINELLI and FURIA 1979), into three different classes depending on the intensity of bobbed phenotype:

bobbed$^+$	= wild type;
slight and intermediate bobbed	= short bristles; normal or slightly etched abdomen;
strong bobbed	= short bristles; strongly etched abdomen.

For every experiment we observed the progeny of six bottles, corresponding to about 3000 flies. In each bottle 10 males were mated to 10 females.

Scoring for the mei9 *mutation:* Presence of the *mei9*^{a} allele was checked by testing for elevated sensitivity to UV radiation (BOYD, GOLINO and SETLOW 1976) and for nondisjunction in homozygous females (BAKER and CARPENTER 1972).

DNA extraction: DNA was extracted from adult flies as described by RITOSSA (1966).

Labeling and RNA extraction: ^{3}H-ribosomal RNA was extracted and purified from wild-type larvae according to BONCINELLI and FURIA (1979). Specific activity was not less than 200,000 cpm/μg.

rRNA/DNA hybridization: rRNA was hybridized to DNA bound to nitrocellulose filter discs, according to GILLESPIE and SPIEGELMAN (1965).

RESULTS

In order to study the effect of the *mei9* mutation on rDNA magnification, a spontaneous *bb* mutant was selected on an X chromosome bearing the *mei9*^{a} allele, as described in Figure 1. The rDNA gene percentage in males of the *mei9*^{a} stock was 0.446%, as estimated by means of ^{3}H rRNA/DNA hybridization, and the rDNA contribution of the Y chromosome, determined in a separate cross (see Table 1) was 0.195%. The rDNA level of the X chromosome bearing the *mei9*^{a} allele was calculated by subtraction to be 0.251%, whereas the rDNA content of the *mei9*^{a} *bb* selected chromosome was 0.099%; thus, the bobbed locus was deleted for about 60% of its wild-type rDNA level. This *mei9*^{a} *bb* chromosome was magnified in combination with a *B*^{s}*Y bb y*$^+$ *l(1)sc*$^{J1+}$ helper chromosome (MALVA *et al.* 1972) according to the scheme described in MATERIALS AND METHODS. Since the efficiency of magnification is known to be inversely correlated with the rDNA redundancy level of *bb* flies (RITOSSA 1976) and the original *B*^{s}*Y bb y*$^+$*l(1)sc*$^{J1+}$ chromosome carries only a small deletion of the bobbed locus, a chromosome carrying a wider deletion was selected from the stock (see MATERIALS AND METHODS). The Y *bb*$^-$ chromosome of SCHULTZ, known to carry very few genes for ribosomal RNA (RITOSSA 1976), could not be used in this magnification scheme, because *mei9*^{a} *bb*/Y *bb*$^-$ males are not viable. The X chromosome of the *mei9*^{a} stock carries at its distal tip a lethal that is suppressed by Y chromosomes bearing an X tip translocation such as the *B*^{s}*Y bb y*$^+$*l(1)sc*$^{J1+}$ chromosome.

The rDNA contribution of the *B*^{s}*Y bb y*$^+$*l(1)sc*$^{J1+}$ chromosome was determined by subtracting from the rDNA content of C(1)RM, *y*^{2}*su w*^{a}*w*^{a}*bb*/*B*^{s}*Y bb y*$^+$*l(1)sc*$^{J1+}$ females the redundancy level of the same females lacking free Y

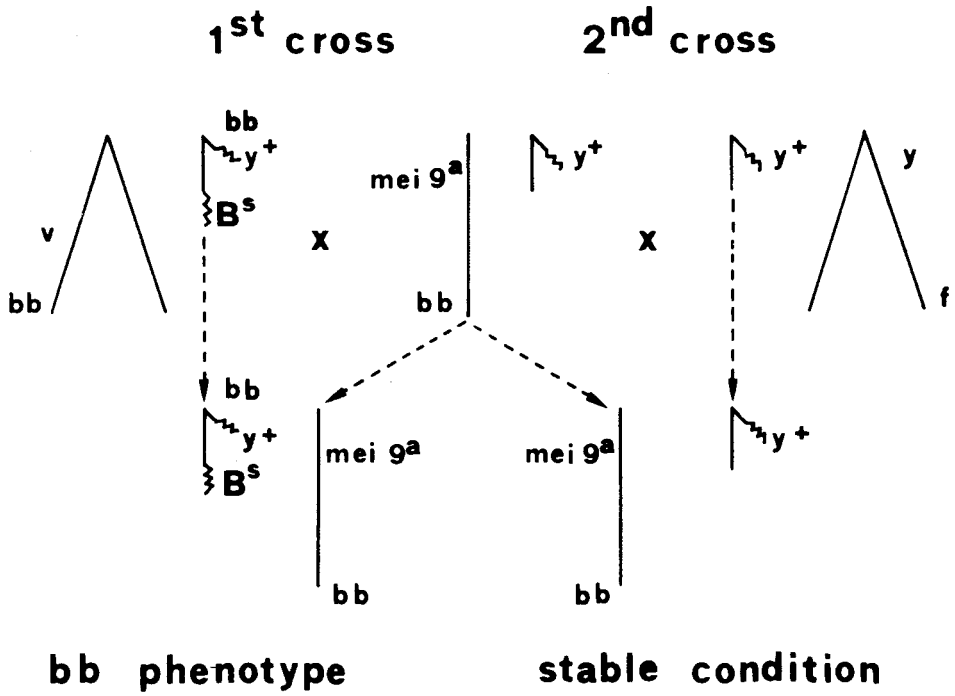

FIGURE 1.—Scheme for the selection of a spontaneous bobbed mutation on the X chromosome bearing the *mei9*^{a} allele : *y mei9*^{a}/*y*$^+$Y males were singly mated to *C(1)RM, l(1)sc*^{J1}*v bb*/*B*^{s}Y *bb y*$^+$*l(1)sc*$^{J1+}$ virgins (first cross). After 4–5 days the same males were singly crossed with *C(1)DX, y f*/*y*$^+$Y females (second cross). The presence of a spontaneous bobbed mutation on the X chromosome bearing *mei9*^{a} was revealed in the first cross by the bb phenotype exhibited by all the male offspring. In the second cross the selected chromosome was kept in a stable condition to avoid variation of rDNA redundancy.

chromosome (see Table 1). *C(1)RM, y f* females, known to lack the NO region (RITOSSA 1976) were not used in order to avoid ambiguous results arising from rDNA increase occurring in *C(1)RM, y f*/Y *bb* bobbed females (GARGANO and GRAZIANI 1976).

Results of the phenotypic analysis of the first eight generations of magnification (*pre* to m_7) of *mei9*^{a} *bb*/*B*^{s}Y *bb y*$^+$*l(1)sc*$^{J1+}$ males are shown in Table 2. The percentages reported are given as variation ranges derived from three independent experiments. At the m_2 generation, when the bb$^+$ phenotype normally has reached full penetrance, a large number of *bb* males is still present (POLITO *et al.* 1980).

The analysis was then extended to m_7, when the magnification process is normally complete; at this stage the males should carry a stable *bb*$^+$ locus that is also no longer capable of reverting. On the contrary, about 50% of the *mei9*^{a} *bb* mutants still showed at this generation a bobbed phenotype, indicating that the meiotic mutation significantly affects the rDNA magnification process. However, since the X chromosome bearing the *mei9*^{a} allele was obtained by EMS treatment (BAKER and CARPENTER 1972), the observed effect might not result from the *mei9*^{a} allele, but rather from some unknown mutation present on that chromosome.

To check this possibility we made the cross described in Figure 2. The progeny of such a cross were scored for recombinant males of the y pn

TABLE 1

Estimation of rDNA content of mei9^a *bb males in various magnification steps*

Genotype	Magnification step	rRNA/DNA percentage[a]			
		Total	X chromosome contribution	Y chromosome contribution	Increase
C(1)RM, y^2*su-w*^{a}*w*^{a} *bb/0*	Established stock	0.262 ± 0.021	0.262	—	—
C(1)RM, y^2*su-w*^{a}*w*^{a} *bb/y*$^+$Y	Established stock	0.457 ± 0.015[b]	0.262	0.195	—
y mei9^a*/y*$^+$Y	Established stock	0.446 ± 0.028	0.251	0.195	—
y mei9^a *bb/y*$^+$ Y	Established stock	0.294 ± 0.014	0.099	0.195	—
C(1)RM, y^2*su-w*^{a}*w*^{a} *bb/ B*^{s}*Ybby*$^+$*l(1)sc*$^{J1+}$	Established stock	0.330 ± 0.019[b]	0.262	0.068	—
y mei9^a *bb/B*^{s}*Ybby*$^+$*l(1)sc*$^{J1+}$	*pre*	0.208 ± 0.018	0.099	0.068	0.041
y mei9^a *bb/B*^{s}*Ybby*$^+$*l(1)sc*$^{J1+}$	m_2	0.215 ± 0.020	0.099	0.068	0.048
y mei9^a *bb/B*^{s}*Ybby*$^+$*l(1)sc*$^{J1+}$	m_7	0.213 ± 0.013	0.099	0.068	0.046
y mei9^a *bb/B*^{s}*Ybby*$^+$*l(1)sc*$^{J1+}$	*pre rev pre*	0.212 ± 0.016	0.099	0.068	0.045
y mei9^a *bb/B*^{s}*Ybby*$^+$*l(1)sc*$^{J1+}$	m_2 *rev pre*	0.209 ± 0.017	0.099	0.068	0.042
y mei9^a *bb/B*^{s}*Ybby*$^+$*l(1)sc*$^{J1+}$	m_7 *rev pre*	0.214 ± 0.015	0.099	0.068	0.047

[a] Values are given as mean ± standard error of a series of at least six independent experiments.
[b] These values have been obtained by multiplying the hydridization data by 1.1 to compensate for the XXY DNA content (RITOSSA 1976).

TABLE 2

Screening of bobbed phenotype during magnification of mei9^{a}bb *males*

Magnification step	Percentage[a]		
	Strong *bb*	Slight and intermediate *bb*	Total *bb*
pre	83–87	3–8	86–95
m_2	30–34	35–41	65–75
m_7	26–30	22–27	48–57

[a] Variation range.

phenotype. The presence of the *bb* mutation on these y pn recombinant chromosomes was tested in single crosses where *y pn* males were mated with *C(1)RM,y v f*/Y *bb*$^-$ females. A bobbed mutation was revealed by a *bb* phenotype in the male offspring (which bears an X recombinant chromosome lacking the lethal at the distal end of the chromosome). X *bb* chromosomes were checked for the presence of the *mei9*^{a} allele and for the level of rDNA redundancy (see MATERIALS AND METHODS). A bobbed chromosome lacking the *mei9*^{a} mutation was magnified both with Y *bb*$^-$ or *B*^{s}Y *bb y*$^+$*l(1)sc*$^{J1+}$ as partner chromosomes in control experiments. The *y pn bb* recombinant chromosome lost the *mei9*^{a} mutation whereas the *bb* deletion was still linked with 92.5–80% of the original EMS treated chromosome. All the *y pn bb*/Y *bb*$^-$ or *y pn bb/B*^{s} Y *bb y*$^+$*l(1)sc*$^{J1+}$ males present at the m_2 generation were phenotypically *bb*$^+$. This observation strongly favors a specific effect of the *mei9* mutation in disturbing the magnification process. In order to analyze at the molecular level this effect we have checked the rDNA content of *mei9*^{a} *bb* magnifying males (see Table 1).

The level of rDNA redundancy at the *pre* generation is higher than the

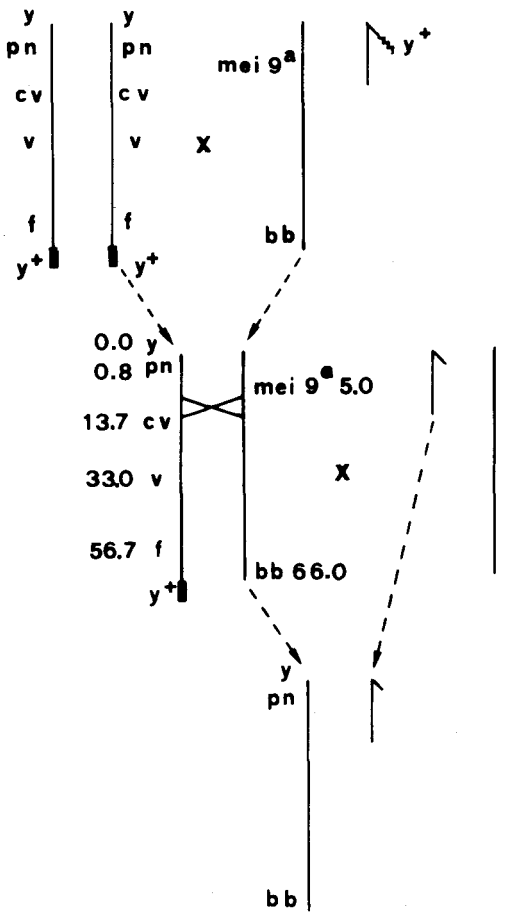

FIGURE 2.—Cross designed to separate the selected bobbed mutation from the *mei9*^{a} allele. Homozygous *y pn cv v f y* females were obtained by suitable crosses starting from the *y pn cv v f* $y^{+}/B^{s}Y$, *C(1)DX*/$B^{s}Y$ stock. Such females were mated to *y mei9*^{a} *bb*/$y^{+}Y$ males and the F_1 heterozygous females were crossed with wild-type Canton S males. Male progeny of the last cross were scored for the recombinant phenotype *y pn*.

expected value calculated by adding X and Y rDNA contributions estimated by separated crosses. This increase seems to indicate that the first step of the magnification process is hardly affected if at all and that synthesis of rDNA extracopies can occur.

It is known that rDNA magnification is a stepwise process, in which there is a progressive increase in rDNA content through succeeding generations until the wild-type level is reached (RITOSSA *et al.* 1971). On the contrary, the rRNA/DNA hybridization data of *mei9*^{a} bb males at m_2 and m_7 generations show that no further increase of rDNA level occurs after the first magnification step. Persistence of the bb^{+} phenotype through the m_7 stage is thus correlated with the absence of additive rDNA synthesis.

It has been stated that reverted *bb* loci behave differently in a second magnification cycle, depending on whether they were at the first stage of magnification or had nearly completed the magnification process at the moment the reversion started; this differential behavior is based on the different amount of extracopies of rDNA integrated at the corresponding stage (BONCINELLI and FURIA 1979). To determine whether this behavior appears also in *mei9* mutants, *pre*, m_2 and m_7 loci were allowed to revert in combination with a B^s Y bb^+ partner chromosome. *pre*, *rev*, m_2*rev* and m_7*rev* loci were then subjected to a second magnification cycle, and their rDNA content was measured. All lines show nearly identical rDNA redundancy values (see Table 1); moreover, these values are approximately equal to the rDNA levels reached at the first magnification round. These observations favor the hypothesis that a defect in the integration of produced rDNA extracopies takes place in *mei9*^{a} mutants.

DISCUSSION

In this paper we report the analysis of the rDNA magnification process in the recombination-deficient *mei9* mutant at the phenotypic and rDNA content levels.

Phenotypic observation of *mei9*^{a} *bb* magnifying males through eight generations demonstrates that these mutants fail to reach bb^+ full penetrance. The fact that the recovery of the bb^+ phenotype is strongly disturbed may be caused by some deficiency in one of the following steps: i) the extra synthesis of rDNA; ii) the functionality of the newly synthesized extracopies and/or the integration process.

i) The rRNA/DNA hybridization data reported show a significant increase of rDNA content at the first generation of magnification in *mei9*^{a} *bb* males. However, no further increase is observed in the next generations, and *bb* loci fail to reach the wild-type redundancy value. This observation clearly indicates that, although the first step of the process is hardly affected if at all, the occurrence of the successive steps are inhibited and the further progress of the magnification is blocked.

ii) The rDNA extracopies produced in the first step of magnification are reported not to be fully functional; in fact, at this stage an accumulation of undermethylated rRNA precursor that seems unable to undergo complete processing has been observed (RITOSSA *et al.* 1971; GRAZIANI and GARGANO 1976). The progressive disappearance of this accumulated rRNA precursor is accompanied in the next generations by full recovery of the bb^+ phenotype (RITOSSA 1976).

As regards the *mei9*^{a} *bb* magnifying males, only a slow increase of the bb^+ phenotype is observed over several generations, suggesting that the extra synthesized genes resume their function poorly. On the other hand, the functionality of the extra genes produced could be related to their integration into the chromosomes. It is known that only newly magnified loci can revert to the *bb* condition and that the capability to revert disappears during the succeeding generations, indicating a progressive integration of the extrachromosomal rRNA genes.

Conversely, no differential behavior as regards the reversion is observed for *mei9*^{a} mutants among *pre*, m_2 and m_7 rDNA loci (data not shown). Moreover,

when *pre rev*, m_2*rev* and m_7*rev* loci from *mei9*^{a} stock were subjected to a second magnification cycle, they reached the same rDNA redundancy value acquired during the first round. These observations suggest that the abortive magnification displayed by *mei9*^{a} mutant could be caused by a deficiency in the integration of the extra synthesized rRNA genes.

In addition to its role in genetic recombination, the product of the *mei9* gene is involved in DNA repair: *mei9* mutants were reported to be defective in repair of DNA damage induced by UV and X rays and they are hypersensitive to killing by these agents (BAKER *et al.* 1976; BOYD, GOLINO and SETLOW 1976; NGUYIEN and BOYD 1977). One of the hypotheses evoked by NGUYIEN and BOYD to account for the close relationship between DNA repair and genetic recombination in *D. melanogaster* is that the *mei9* mutants could be defective in a nuclease or in a factor controlling the expression of this nuclease.

Previously reported data (PEPE AND POLITO 1975) pointed out that, in testes of magnifying males, an increase in the DNAse activity occurs, suggesting a role for this activity during magnification. Therefore, one can assume that the signal, at least for the extracopy integration, could involve breaks in the chromosomal rDNA and that the increased DNAse level could be responsible for the generation of such breaks. On the other hand, the integration could be an essential step also in regard to the progress of the process and its accomplishment capacity. Our results suggest that an enzymatic activity, or a factor controlling its expression, is involved in both repair-recombination and rDNA magnification processes.

An alternative model based on unequal mitotic sister chromatid exchange was proposed to account for rDNA magnification (TARTOF 1974). According to this model, rDNA magnification could be a premeiotic event. However, since the level of mitotic sister chromatid exchange is reported to be only slightly affected in *mei9* flies (GATTI, PIMPINELLI and BAKER 1980), the abortive magnification observed in *mei9*^{a} can hardly be explained on this basis. Nevertheless, a selective mechanism based on unequal mitotic sister chromatid exchange could contribute to the *bb*$^+$ phenotypical recovery when the normal pathway of the rDNA magnification is inhibited. A stimulating hypothesis to interpret the slow increase of *bb*$^+$ percentage observed in *mei9*^{a} mutants between *pre* and m_7 stages, having the same rDNA content might, in fact, be based on a rectification process (SMITH 1973) which could eliminate from the locus genes with intervening sequences that are known to be hardly and unproductively transcribed also in *bobbed* mutants (LONG *et al.* 1981).

We are indebted to BRUCE BAKER who generously made available for us *y mei9*^{a}/*y*$^+$*Y, C(1)DX, y f*/*y*$^+$*Y; spa*^{pal}/*spa*^{pal} and *y pn cv v f y*$^+$/*B*^{s}*Y, C(1)DX*/*B*^{s}*Y* stocks used in this work. We thank GIOVANNI IMPERATO for technical assistance.

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Corresponding editor: M. L. PARDUE